REMARKS

The Examiner is respectfully requested to provide a Notice of References Cited Form PTO-892 which lists Clive et al. which was applied in a prior art rejection in the April 25, 2001 Office Action.

Claim 1 was amended to include features of claim 41.

Claims 5 and 43 were editorially revised.

Claim 39 was amended to delete subject matter.

The dependency of claim 42 was changed.

Claims 2 to 8 and 39 to 43 were rejected under 35 USC 112, second paragraph, for the reasons set forth in Paragraph No. 3 on pages 2 to 3 of the Office Action.

Claims 2, 5 and 7 were amended to avoid the 35 USC 112, second paragraph rejection. Claim 8 was amended in a manner similar to claim 7.

Enclosed is a MARKED-UP VERSION OF THE AMENDMENTS TO THE CLAIMS.

It is respectfully submitted that the present claims comply with all the requirements of 35 USC 112. Withdrawal of the 35 USC 112, second paragraph rejection is therefore respectfully solicited.

Claims 1 to 8 and 39 were rejected under 35 USC 102 as being anticipated by Eigen et al. USP 5,807,677 for the reasons set forth in Paragraph No. 5 bridging pages 3 and 4 of the Office Action.

Claim 40 was rejected under 35 USC 103 as being unpatentable over Eigen et al. in view of Gyllensten et al., Proc. National Academic Science USA, 85, 7652-7656, (1988) for the reasons set forth in Paragraph No. 7 bridging pages 4 and 5 of the Office Action.

Claims 41 to 43 were deemed to be free of any prior art rejection as set forth in Paragraph No. 8 at the middle of page 5 of the Office Action.

The incorporation of features of claim 41 into claim 1 should serve to make all the present claims free of any prior art rejection.

The present invention as recited in claim 1 is directed to a method of analyzing a target nucleic acid by applying a nucleic acid amplification reaction to a test solution, wherein an amplified product is labeled with a marker molecule. An important feature of the present invention resides in that the number of one of the forward primer and the reverse primer is lower than that of the other primer, and the primer present in a lower number is labeled with a marker molecule capable of

generating a detectable signal. According to applicant's method, an amplified double stranded nucleic acid product is labeled with the marker molecule without fail, whereas a single stranded nucleic acid product is not labeled (see Fig. 3 of the present application). The reaction is observed by fluorescence correlation spectroscopy ("FCS"). In this manner, the present invention makes it possible to analyze a target nucleic acid.

In contrast to the presently claimed invention, neither of the two references cited in the above-described rejections disclose the aforementioned important feature of the presently claimed invention.

Eigen et al. disclose FCS observation by labeling a PCR product with a fluorescence marker. The Office Action admitted that Eigen et al. do not disclose using asymmetric nucleic acid amplification.

Gyllensten et al. do not disclose a FCS observation performed by introducing a fluorescence marker into an amplified product. Hence, Gyllensten et al. do not teach or suggest the feature of a "primer present in a lower number is labeled with a marker molecule".

It is respectfully submitted that one of ordinary skill in the art would not consider combining the two references. However, assuming *arguendo* that the two references are combined,

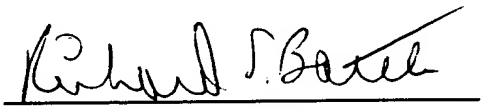
the feature of the present invention of "the number of one of the forward primer and the reverse primer is lower than that of the other primer, and the primer present in a lower number is labeled with a marker molecule capable of generating a detectable signal" would not be arrived at.

It is therefore respectfully submitted that applicant's claimed invention is not anticipated and is not rendered obvious over the references, either singly or combined in the manner relied upon in the Office Action in view of the distinctions discussed hereinabove. It is furthermore submitted that there are no teachings in the references to combine them in the manner relied upon in the Office Action.

Reconsideration is requested. Allowance is solicited.

If the Examiner has any comments, questions, objections or recommendations, the Examiner is invited to telephone the undersigned at the telephone number given below for prompt action.

Respectfully submitted,



RICHARD S. BARTH
REG. NO. 28,180

FRISHAUF, HOLTZ, GOODMAN & CHICK, P.C.
767 THIRD AVENUE - 25TH FLOOR
NEW YORK, NEW YORK 10017-2023
Tel. No. (212) 319-4900
Fax No. (212) 319-5101
RSB/ddf

Encs.: (1) PETITION FOR EXTENSION OF TIME
(2) MARKED-UP VERSION OF THE AMENDMENTS TO THE CLAIMS

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MARKED-UP VERSION OF THE AMENDMENTS TO THE CLAIMS
(Serial No. 09/325,189)

1. (Thrice Amended) A method of analyzing a target nucleic acid by applying a nucleic acid amplification reaction to a test solution, wherein an amplified product is labeled with a marker molecule, said method comprises:

(a) performing a nucleic acid amplification reaction of the target nucleic acid [to provide nucleic acid amplification products including amplified nucleic acid] in a test solution containing a forward primer and a reverse primer, a substrate comprising nucleotides, [wherein at least one of said nucleotides is labeled with a marker molecule capable of generating a detectable signal,] a nucleic acid polymerase[,] and a target nucleic acid molecule, and wherein the number of one of the forward primer and the reverse primer is lower than that of the other primer, and the primer present in a lower number is labeled with a marker molecule capable of generating a detectable signal;

(b) measuring a signal from the marker molecule in the test solution after initiation of the nucleic acid amplification reaction;

(c) evaluating a fluctuation motion of the amplified nucleic acid which is labeled with the marker molecule, in the test solution on the basis of the signal detected; and

(d) quantifying the target nucleic acid molecule on the basis of evaluation results.

2. (Twice Amended) A method according to claim 1, wherein the measurement step includes a step of measuring an amount of the marker molecule present in a predetermined micro detection field, said marker molecule being the primer attached to the target nucleic acid.

5. (Twice Amended) A method according to claim 4, wherein the step of converting includes a step of performing an arithmetic operation by an autocorrelation function.

7. (Twice Amended) A method according to any one of claims 1 to 5, wherein the quantifying of the target nucleic [and] acid molecule includes determining the presence and absence of the marker molecule of the primer attached to the target nucleic acid and incorporated into [the nucleic acid amplification] products [produced during] of the nucleic acid amplification reaction on the basis of the evaluation results.

8. (Twice Amended) The method according to any one of claims 1 to 5, wherein the quantifying of the target nucleic acid molecule includes determining the number of the labeled primer attached to the target nucleic acid and incorporated into [the nucleic acid amplification] products [during] of the nucleic acid amplification reaction on the basis of the evaluation results.

39. (Amended) A method according to any one of claims 1 to 5, wherein [at least one of the forward primer and the reverse primer is labeled with a detectable marker molecule, and at least] the number of labeled primer molecules [being] <u>is</u> known.

42. (Amended) A method according to claim [41] <u>1</u>, wherein the mixing ratio of the forward primer and the reverse primer [in fluid] is in a range of 2:1 to 20:1.

43. (Amended) A method according to claim 42, wherein the mixing [concentration] ratio of the forward primer and the reverse primer [in fluid] is in a range of 800nM:400nM to 800nM:40nM.